

WHAT IS CLAIMED IS:

1 1. A heterofunctional crosslinking reagent having the formula:



2 wherein

3 W is a covalent core component;

4 L^1 , L^2 and L^3 are each independently linking groups;

5 X is a reversibly covalent or non-covalent protein tag binder;

6 Y is a activatable covalent crosslinking group; and

7 Z is a protected or unprotected chemical crosslinking group.

1 2. A heterofunctional crosslinking reagent of claim 1, wherein said
2 core component is a member selected from the group consisting of an amino acid, a
3 sugar, a substituted aromatic ring, a substituted amine, a phosphine or phosphite
4 derivative, and a substituted hydrocarbon.

1 3. A heterofunctional crosslinking reagent of claim 1, wherein said
2 core component is a substituted hydrocarbon or an amino acid selected from the group
3 consisting of lysine, arginine, serine, cysteine, glutamic acid, aspartic acid and threonine.

1 4. A heterofunctional crosslinking reagent of claim 1, wherein L^1 , L^2
2 and L^3 are each independently selected from the group consisting of a bond, a substituted
3 or unsubstituted (C_2 - C_{24})alkylene group, a substituted or unsubstituted (C_2 -
4 C_{24})heteroalkylene group, a polyethyleneglycol group, a polyalcohol group, a polyamine
5 group, a polyester group and a polyphosphodiester group.

1 5. A heterofunctional crosslinking reagent of claim 1, wherein L^1 is a
2 cleavable linking group.

1 6. A heterofunctional crosslinking reagent of claim 5, wherein L^1
2 comprises a disulfide moiety.

1 7. A heterofunctional crosslinking reagent of claim 5, wherein L^1
2 comprises is a disulfide moiety; L^2 and L^3 are each independently selected from the group

3 consisting of a bond, a substituted or unsubstituted (C₂-C₂₄)alkylene group, a substituted
4 or unsubstituted (C₂-C₂₄)heteroalkylene group, a polyethyleneglycol group, a polyalcohol
5 group, a polyamine group, a polyester group and a polyphosphodiester group; Y is a
6 member selected from the group consisting of aryl ketones, azides, diazo compounds,
7 diazirenes, and ketenes; Z is a protected or unprotected member selected from the group
8 consisting of acyl hydrazines, olefins, dicarbonyl groups, epoxides, aldehydes,
9 organosilanes, reactive esters, isocyanates, thioisocyanates, carboxylic acid chlorides,
10 disulfides, sulfonate esters and sulfhydryl groups.

1 **8.** A heterofunctional crosslinking reagent of claim 5, wherein X is
2 selected from the group consisting of TAR, a DNA sequence that specifically binds a
3 homeodomain, a DNA or RNA sequence that specifically recognizes a peptide affinity
4 tag, a leucine zipper helical peptide, a PDZ domain and calmodulin; L¹ comprises a
5 disulfide moiety; L² and L³ are each independently selected from the group consisting of
6 a bond, a substituted or unsubstituted (C₂-C₂₄)alkylene group, a substituted or
7 unsubstituted (C₂-C₂₄)heteroalkylene group, a polyethyleneglycol group, a polyalcohol
8 group, a polyamine group, a polyester group and a polyphosphodiester group; Y is a
9 member selected from the group consisting of aryl ketones, azides, diazo compounds,
10 diazirenes, and ketenes; and Z is a protected or unprotected member selected from the
11 group consisting of acyl hydrazines, olefins, dicarbonyl groups, epoxides, aldehydes,
12 organosilanes, reactive esters, isocyanates, thioisocyanates, carboxylic acid chlorides,
13 disulfides, sulfonate esters and sulfhydryl groups.

1 **9.** A heterofunctional crosslinking reagent of claim 1, wherein X is
2 selected from the group consisting of metal chelating groups, peptides, an organoarsenical
3 moiety and small molecule ligands or inhibitors.

1 **10.** A heterofunctional crosslinking reagent of claim 1, wherein Y is a
2 member selected from the group consisting of aryl ketones, azides, diazo compounds,
3 diazirenes, and ketenes.

1 **11.** A heterofunctional crosslinking reagent of claim 1, wherein Z-L³-
2 is an amino acid side chain having a pendant reactive group, wherein said amino acid is
3 selected from the group consisting of lysine, cysteine, serine, aspartic acid, glutamic acid
4 and threonine.

1 **12.** A heterofunctional crosslinking reagent of claim 1, wherein Z is a
2 protected or unprotected member selected from the group consisting of acyl hydrazines,
3 olefins, dicarbonyl groups, epoxides, aldehydes, reactive esters, isocyanates,
4 thioisocyanates, carboxylic acid chlorides, dissulfides, sulfonate esters and sulphydryl
5 groups.

1 **13.** A heterofunctional crosslinking reagent of claim 1, wherein W is a
2 member selected from the group consisting of an amino acid, a sugar, a substituted
3 aromatic ring, a substituted amine, a phosphine or phosphite derivative, and a substituted
4 hydrocarbon; L¹, L² and L³ are each independently selected from the group consisting of
5 a bond, a (C₂-C₂₄)alkylene group, a polyethyleneglycol group, a polyalcohol group, a
6 polyamine group, a polyester group and a polyphosphodiester group; X is selected from
7 the group consisting of a metal chelating group, a peptide and a small molecule ligand or
8 inhibitor; Y is selected from the group consisting of aryl ketones, azides, diazo
9 compounds, diazirenes, and ketenes; Z is selected from the group consisting of acyl
10 hydrazines, olefins, acetylenes, dicarbonyl groups, epoxides, aldehydes, reactive esters,
11 isocyanates, thioisocyanates, carboxylic acid chlorides, dissulfides, sulfonate esters and
12 sulphydryl groups.

1 **14.** A heterocrosslinking compound having the formula:



3 wherein

4 L is a helical component of a leucine zipper;
5 L¹ and L² are each independently selected from the group consisting of a bond and
6 a linking group;
7 Y is a activatable covalent crosslinking group; and
8 Z is a protected or unprotected chemical crosslinking group.

1 **15.** A crosslinking compound of claim 14, wherein L is a helical
2 peptide having from about 12 to about 50 amino acid residues.

1 **16.** A crosslinking compound of claim 14, wherein L is a helical
2 peptide having from about 12 to about 50 amino acid residues, and Y is a

3 photocrosslinking group selected from the group consisting of aryl ketones, azides, diazo
4 compounds, diazirenes, and ketenes.

1 **17.** A crosslinking compound of claim 14, wherein L is a helical
2 peptide having from about 12 to about 50 amino acid residues; Y is a photocrosslinking
3 group selected from the group consisting of aryl ketones, azides, diazo compounds,
4 diazirenes, and ketenes; and Z is a protected or unprotected reactive functional group
5 selected from the group consisting of acyl hydrazines, olefins, dicarbonyl groups,
6 epoxides, aldehydes, reactive esters, isocyanates, thioisocyanates, carboxylic acid
7 chlorides, dissulfides, sulfonate esters and sulphydryl groups.

1 **18.** A crosslinking compound of claim 14, wherein L¹ and L² are other
2 than a bond, and Y and Z are independently attached to L, through said L¹ and said L², to
3 the same or different amino acids within three residues of the N-terminus of said helical
4 peptide.

1 **19.** A crosslinking compound of claim 14, wherein L¹ and L² are other
2 than a bond, and Y and Z are independently attached to L, through said L¹ and said L², to
3 the same or different amino acids within three residues of the C-terminus of said helical
4 peptide.

1 **20.** A crosslinking compound of claim 14, wherein L¹ and L² are other
2 than a bond, and Y and Z are independently attached to L, through said L¹ and said L², to
3 amino acids within three residues of different termini of said helical peptide.

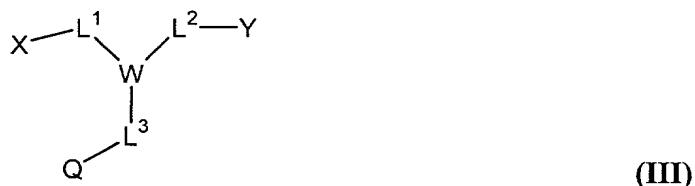
1 **21.** A crosslinking compound of claim 18, wherein L is a helical
2 peptide having from about 12 to about 50 amino acid residues; Y is a photocrosslinking
3 group selected from the group consisting of aryl ketones, azides, diazo compounds,
4 diazirenes, and ketenes; and Z is a protected or unprotected reactive functional group
5 selected from the group consisting of acyl hydrazines, olefins, dicarbonyl groups,
6 epoxides, aldehydes, reactive esters, isocyanates, thioisocyanates, carboxylic acid
7 chlorides, dissulfides, sulfonate esters and sulphydryl groups.

1 **22.** A crosslinking compound of claim 19, wherein L is a helical
2 peptide having from about 12 to about 50 amino acid residues; Y is a photocrosslinking
3 group selected from the group consisting of aryl ketones, azides, diazo compounds,

4 diazirenes, and ketenes; and Z is a protected or unprotected reactive functional group
5 selected from the group consisting of acyl hydrazines, olefins, dicarbonyl groups,
6 epoxides, aldehydes, reactive esters, isocyanates, thioisocyanates, carboxylic acid
7 chlorides, dissulfides, sulfonate esters and sulphydryl groups.

1 23. A crosslinking compound of claim 20, wherein L is a helical
2 peptide having from about 12 to about 50 amino acid residues; Y is a photocrosslinking
3 group selected from the group consisting of aryl ketones, azides, diazo compounds,
4 diazirenes, and ketenes; and Z is a protected or unprotected reactive functional group
5 selected from the group consisting of acyl hydrazines, olefins, dicarbonyl groups,
6 epoxides, aldehydes, reactive esters, isocyanates, thioisocyanates, carboxylic acid
7 chlorides, dissulfides, sulfonate esters and sulphydryl groups.

1 **24.** A protein labeling reagent having the formula:



2 wherein

3 W is a covalent core component;
4 L^1 , L^2 and L^3 are each independently linking groups;
5 X is a reversibly covalent or non-covalent protein tag binder;
6 Y is a activatable covalent crosslinking group; and
7 Q is a label or a reporter group.

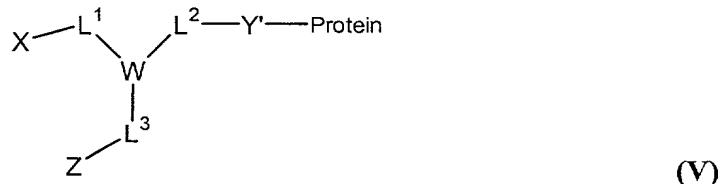
1 25. A protein labeling reagent having the formula:



3 wherein

4 L is a helical component of a leucine zipper;
5 L^1 and L^2 are each independently selected from the group consisting of a bond and
6 a linking group;
7 Y is a activatable covalent crosslinking group; and
8 Q is a label or a reporter group.

1 **26.** A protein conjugate comprising a protein and a heterofunctional
2 crosslinking reagent, said conjugate having the formula:



3 wherein

4 W is a covalent core component;
5 L¹, L² and L³ are each independently linking groups;
6 X is a reversibly covalent or non-covalent protein tag binder;
7 Y' is the residue of a activatable covalent crosslinking group after formation of a
8 covalent linkage to said protein; and
9 Z is a protected or unprotected covalent crosslinking group.

1 **27.** A protein-conjugate comprising a protein and a covalently attached
2 heterofunctional linking group of claim 1.

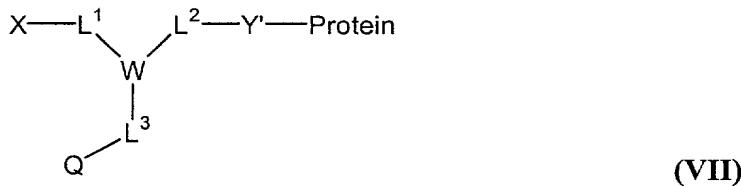
1 **28.** A protein conjugate comprising a protein and a crosslinking
2 reagent, said conjugate having the formula:



4 wherein

5 L is a helical component of a leucine zipper;
6 L¹ and L² are each independently selected from the group consisting of a bond and
7 a linking group;
8 Y' is a photocrosslinking group that has been activated and covalently attached to
9 a protein; and
10 Z is a protected or unprotected chemical crosslinking group.

1 **29.** A protein composition having the formula:



2 wherein

3 W is a covalent core component;
4 L^1 is a linking group;
5 L^2 and L^3 are each independently a bond or a linking group;
6 X is hydrogen or a protein tag binder;
7 Y' is a crosslinking group that has been activated and covalently attached to a
8 protein; and
9 Q is a label or a solid support.

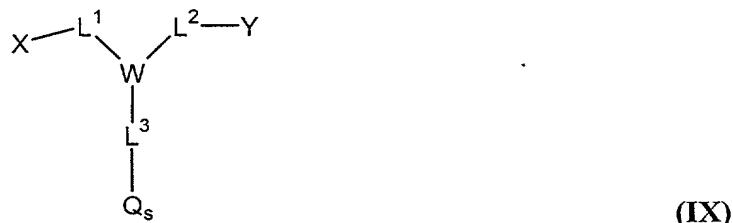
1 30. A protein composition having the formula:



2 wherein

3 L is a helical component of a leucine zipper;
4 L^1 and L^2 are each independently selected from the group consisting of a bond and
5 a linking group;
6 Y' is a crosslinking group that has been activated and covalently attached to a
7 protein; and
8 Q is a solid support or a label.

1 31. A support-bound crosslinking group having the formula:



2 wherein

3 W is a covalent core component;
4 L^1 , L^2 and L^3 are each independently linking groups;
5 X is a reversibly covalent or non-covalent protein tag binder;
6 Y is a activatable covalent crosslinking group; and
7 Q_s is a member selected from the group consisting of a solid support, a monolayer
8 attached to a support and a thinfilm attached to a support.

1 32. A modified support having the formula:



3 wherein

4 L is a helical component of a leucine zipper;

5 L¹ and L² are each independently selected from the group consisting of a bond and
6 a linking group;

7 Y is a activatable covalent crosslinking group; and

8 Q_s is a member selected from the group consisting of a solid support, a monolayer
9 attached to a support and a thinfilm attached to a support.

1 33. A protein array comprising:

2 (a) a substrate;

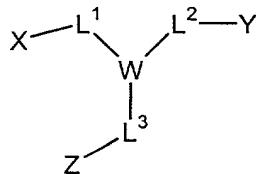
3 (b) at least one organic thinfilm on at least a portion of the substrate

4 surface; and

5 (c) a plurality of patches arranged in discrete, known regions on portions
6 of the substrate surface covered by said organic thinfilm, wherein each of said patches
7 comprises a protein immobilized on the underlying organic thinfilm by a heterofunctional
8 crosslinking agent.

1 34. A protein array in accordance with claim 33, wherein said

2 heterofunctional crosslinking agent has the formula:



3 wherein

4 W is a covalent core component;

5 L^1, L^2 and L^3 are independently linking groups;

6 X is a reversibly covalent or non-covalent protein tag binder;

7 Y is a activatable covalent crosslinking group; and

8 Z is a protected or unprotected covalent crosslinking group.

1 **35.** A protein array in accordance with claim 33, comprising at least 10
2 patches.

1 **36.** A protein array in accordance with claim 33, comprising at least
2 100 patches.

1 **37.** A protein array in accordance with claim 33, comprising at least
2 1000 patches.

1 **38.** A protein array in accordance with claim 33, comprising at least 10
2 different immobilized proteins.

1 **39.** A protein array in accordance with claim 33, comprising at least
2 100 different immobilized proteins.

1 **40.** A protein array in accordance with claim 33, comprising at least
2 1000 different immobilized proteins.

1 **41.** A protein array in accordance with claim 33, wherein the area of
2 the substrate surface covered by each of the patches is no more than about 0.25 mm².

1 **42.** A protein array in accordance with claim 33, wherein the area of
2 the substrate surface covered by each of the patches is from about 1 µm² to about 10,000
3 µm².

1 **43.** A protein array in accordance with claim 33, wherein the proteins
2 immobilized on the array are all functionally related.

1 **44.** A protein array in accordance with claim 33, wherein the proteins
2 immobilized on the array are all structurally related.

1 **45.** A method for attaching a protein to a solid support, said method
2 comprising:

3 (i) forming a reversibly covalent or non-covalent association between
4 said protein and a protein tag binder of a heterofunctional crosslinking reagent;
5 (ii) forming a covalent linkage between said solid support and a first
6 functional group of said heterofunctional crosslinking reagent; and
7 (iii) forming a covalent linkage between said protein and a second
8 functional group of said heterofunctional crosslinking reagent.

1 **46.** A method for covalently attaching to a protein a heterofunctional
2 crosslinking reagent having an available functionalized linker arm, said method
3 comprising:

4 (i) forming a non-covalent association between said protein and a
5 protein tag binder present on a heterofunctional crosslinking reagent;
6 (ii) forming a covalent linkage between said protein and a first reactive
7 functional group of said heterofunctional crosslinking reagent, to provide a protein having
8 a covalently attached heterofunctional crosslinking reagent having an available
9 functionalized linker arm.

1 **47.** A method for covalently attaching a heterofunctional crosslinking
2 reagent to a recombinant protein having an engineered helical portion, said method
3 comprising:

4 (i) forming a non-covalent association complex between said
5 engineered helical portion of said recombinant protein and a heterofunctional crosslinking
6 reagent comprising a peptide helical portion, a second covalent crosslinking group and a
7 third crosslinking group that is unreactive to functional groups normally present on a
8 protein; and

9 (ii) activating said non-covalent association complex to form a
10 covalent linkage between said recombinant protein and said second crosslinking group of
11 said heterofunctional crosslinking reagent.

1 **48.** A method in accordance with claim 47, wherein said engineered
2 helical portion is a first component of a leucine zipper.

1 **49.** A method in accordance with claim 48, wherein said first
2 component of a leucine zipper comprises at least two leucine residues.

1 **50.** A method in accordance with claim 48, wherein said first
2 component of a leucine zipper comprises at least four leucine residues.

1 **51.** A method in accordance with claim 47, wherein said
2 photocrosslinking group is selected from the group consisting of aryl ketones, azides,
3 diazo compounds, diazirenes, and ketenes.

1 **52.** A method in accordance with claim 47, wherein said peptide
2 helical portion is a second component of a leucine zipper comprising of from 15 to about
3 50 amino acids, said photocrosslinking group is selected from the group consisting of aryl
4 ketones, azides, diazo compounds, diazirenes, and ketenes, and said chemical
5 crosslinking portion is a reactive functional group optionally having an attached
6 protecting group.

1 **53.** A method for attaching a crosslinking reagent to a recombinant
2 protein having an engineered helical peptide portion comprising at least four cysteine
3 residues, said method comprising:

4 (i) forming a covalent complex between said engineered helical
5 peptide portion of said recombinant protein and a heterofunctional crosslinking reagent,
6 wherein said heterofunctional crosslinking reagent comprises an organoarsenical group
7 reactive with said at least four cysteine residues present in said engineered helical peptide
8 portion, a photocrosslinking portion and a chemical crosslinking portion that is unreactive
9 to functional groups normally present on a protein;
10 (ii) activating said covalent complex to form a covalent linkage
11 between said recombinant protein and said photocrosslinking group of said
12 heterofunctional crosslinking reagent; and
13 (iii) releasing said organoarsenical group from said protein and said
14 crosslinking reagent to provide a recombinant protein having an attached crosslinking
15 reagent.

1 **54.** A method in accordance with claim 53, wherein said engineered
2 helical peptide portion of said recombinant protein comprises from about 20 to about 50
3 amino acid residues.

1 **55.** A method in accordance with claim 53, wherein said
2 photocrosslinking group is selected from the group consisting of aryl ketones, azides,
3 diazo compounds, diazirenes, and ketenes.

1 **56.** A method in accordance with claim 53, wherein said engineered
2 helical peptide portion comprises from about 20 to about 50 amino acids, said
3 photocrosslinking group is selected from the group consisting of aryl ketones, azides,

4 diazo compounds, diazirenes, and ketenes, and said chemical crosslinking portion is a
5 reactive functional group having an attached protecting group.

1 **57.** A method in accordance with claim **53**, wherein said engineered
2 helical peptide portion comprises from about 20 to about 50 amino acids, said
3 photocrosslinking group is selected from the group consisting of aryl ketones, azides,
4 diazo compounds, diazirenes and ketenes; said chemical crosslinking portion is a reactive
5 functional group having an attached protecting group; and said organoarsenical group
6 comprises two arsenic atoms attached to a tricyclic hydrocarbon moiety.

1 **58.** A method in accordance with claim **57**, wherein said tricyclic
2 hydrocarbon moiety is a phenanthrene moiety.

1 **59.** A method for attaching a crosslinking reagent to a recombinant
2 protein having a protein affinity tag, said method comprising:

3 (i) forming a non-covalent association complex between said
4 recombinant protein tag and a heterofunctional crosslinking reagent, wherein said
5 heterofunctional crosslinking reagent comprises:

6 (a) a protein tag binder group specific for said protein tag and
7 attached to the remainder of said heterofunctional
8 crosslinking reagent via a cleavable linking group;
9 (b) a covalent crosslinking group; and
10 (c) a protected or unprotected chemical crosslinking group that is
11 unreactive to functional groups normally present on a
12 protein;

13 (ii) activating said non-covalent association complex to form a
14 covalent linkage between said recombinant protein and said covalent crosslinking group
15 of said heterofunctional crosslinking reagent; and

16 (iii) releasing said protein tag binder portion from said heterofunctional
17 crosslinking reagent to provide a recombinant protein having an attached reactive
18 functional group.

1 **60.** A method in accordance with claim **59**, wherein said protein tag is
2 selected from the group consisting of Tat, substance P or a peptide recognized by a

3 specific RNA or DNA aptamer, a DNA-binding homeodomain, a PDZ-binding peptide, a
4 leucine zipper helical peptide and a calmodulin-binding peptide.

1 **61.** A method in accordance with claim **59**, wherein said protein tag
2 binder portion is selected from the group consisting of TAR, a DNA sequence that
3 specifically binds a homeodomain, a DNA or RNA sequence that specifically recognizes
4 a peptide affinity tag, a leucine zipper helical peptide, a PDZ domain and calmodulin.

1 **62.** A method in accordance with claim **59**, wherein said
2 photocrosslinking portion is selected from the group consisting of an aryl ketone, an
3 azide, a diazo compound, a diazirene and a ketene.

1 **63.** A method in accordance with claim **59**, wherein said chemical
2 crosslinking portion is selected from the group consisting of an acyl hydrazine, an olefin,
3 a dicarbonyl group, an epoxide, an aldehyde, an organosilane, a reactive ester, an
4 isocyanate, a thioisocyanate, a carboxylic acid chloride, a disulfide, a sulfonate ester and
5 a sulphydryl group.

1 **64.** A method in accordance with claim **59**, wherein said affinity tag is
2 selected from the group consisting of Tat, substance P or a peptide recognized by a
3 specific RNA or DNA aptamer, a DNA-binding homeodomain, a PDZ-binding peptide, a
4 leucine zipper helical peptide and a calmodulin-binding peptide; said presenting
5 macromolecule portion is selected from the group consisting of TAR, a DNA sequence
6 that specifically binds a homeodomain, a DNA or RNA sequence that specifically
7 recognizes a peptide affinity tag, a leucine zipper helical peptide, a PDZ domain and
8 calmodulin; said photocrosslinking portion is selected from the group consisting of an
9 aryl ketone, an azide, a diazo compound, a diazirene and a ketene, said chemical
10 crosslinking portion is selected from the group consisting of an acyl hydrazine, an olefin,
11 a dicarbonyl group, an epoxide, an aldehyde, an organosilane, a reactive ester, an
12 isocyanate, a thioisocyanate, a carboxylic acid chloride, a disulfide, a sulfonate ester and
13 a sulphydryl group.

1 **65.** A method for attaching an altering member to a polypeptide, the
2 method comprising:

3 a) contacting said polypeptide with said altering member to form a
4 chemically specific, non-covalent complex having a polypeptide component and an
5 altering component;

6 b) providing conditions sufficient to form a covalent bond between said
7 polypeptide component and said altering component;

8 wherein the functional groups taking part in complex formation and in
 9 covalent bond formation are different and step b) is subsequent to step a).

1 **66.** A method of claim **65**, wherein said covalent bond is formed via a
2 radical reaction.

1 **67.** A method of claim **66**, wherein said radical reaction is initiated by
2 white light.

1 **68.** A method for covalently linking a protein to a compound,
2 biological moiety, or substrate within one or more specific regions of said protein, said
3 method comprising the steps of:

4 i) providing a heterofunctional crosslinker comprising;

5 a) one or more first functional groups capable of reversibly covalently or
6 non-covalently crosslinking specifically at one or more first functional
7 group sites within at least one of said one or more specific regions of
8 said protein,

15 d) a covalent core for covalently linking said first, second, and third
16 groups together to form said heterofunctional crosslinking reagent to
17 covalently link said protein, through said heterofunctional crosslinker's
18 covalent core to said compound, biological moiety, or substrate;

19 ii) crosslinking at least one of said one or more first functional groups to at
20 least one of said one or more first functional group sites within said one or
21 more specific regions of said protein;

- iii) selectively crosslinking at least one of said one or more second functional groups within said specific regions of said protein by selectively activating said second functional groups; and,
- iv) selectively crosslinking at least one of said one or more third functional groups to said compound, biological moiety, or substrate;

wherein said protein is covalently linked to said compound, biological moiety, or substrate through said covalent core of said heterofunctional crosslinker.

69. The method of claim 68, wherein said steps (ii) (iii) and (iv) are switched in order as (i) then (iv) then (ii) then (iii).

70. The method of claim 68, wherein at least one of said one or more second functional groups is selected from the group consisting of a biotin, a leucine zipper, a monomer unit of a coiled-coil dimer, a fragment of an antibody, a chelatable metal, and an aptamer.

71. The method of claim 68, wherein at least one of said one or more second functional groups also functions as said covalent core to covalently link at least one of said first functional groups and at least one of said second functional groups together.

72. The method of claim 68, wherein at least one of said one or more second functional groups is a photocrosslinker, and said selectively activating is selectively exposing said second functional group photocrosslinker to a photon source.

73. A heterofunctional crosslinker for covalently linking a protein to a compound, biological moiety, or substrate within one or more specific regions of said protein, said heterofunctional crosslinker comprising

- i) one or more first functional groups capable of reversibly covalently or non-covalently crosslinking specifically at one or more first functional group sites within at least one of said one or more specific regions of said protein,
- ii) one or more second functional groups capable of selectively covalently crosslinking to said protein at or adjacent said first functional group sites when activated under selectively activating conditions,

- iii) one or more third functional groups capable of covalently attaching, chemisorbing, or physisorbing to said compound, biological moiety, or substrate, and,
- iv) a covalent core for covalently linking said first, second, and third groups together to form said heterofunctional crosslinking reagent to covalently link said protein, through said heterofunctional crosslinker's covalent core to said compound, biological moiety, or substrate;

wherein said heterofunctional crosslinker is adapted to covalently link said protein to said compound, biological moiety, or substrate through said covalent core of said heterofunctional crosslinker when at least one of said one or more second functional groups is attached to said protein within at least one of said one or more specific regions, and at least one of said third functional groups is attached to said compound, biological moiety, or substrate.

74. The heterofunctional crosslinker of claim 73, wherein at least one of said one or more second functional groups is selected from the group consisting of a biotin, a leucine zipper, a monomer unit of a coiled-coil dimer, a fragment of an antibody, a chelatable metal, and an aptamer..

75. The heterofunctional crosslinker of claim 73, wherein at least one of said one or more second functional groups also functions as said covalent core to covalently link at least one of said first functional groups and at least one of said second functional groups together.

76. The heterofunctional crosslinker of claim 73, wherein at least one of said one or more second functional groups also functions as said covalent core to covalently link at least one of said first functional groups and at least one of said second functional groups together.

77. The heterofunctional crosslinker of claim 73, wherein at least one of said one or more second functional groups is a photocrosslinker, and said selective activation is selective exposure of said second functional group photocrosslinker to a photon source.